APPENDIX 5

PCR Protocols
Methods and Applications
Edited by
avid H. Gelfand, John J. Sninsky, and
Thomas J. White

in reaction (PCR) is a powerful new
method with widespread applications in
diagnosis. With over fifty chapters of
this unique, comprehensive benchtop
complete range of PCR methods and
Equipment, reagents, and supplies are

features include:
hensive study of PCR protocols, from
ltiplex PCR methodology
es, with details on reagents and equip-

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ding contamination

is B. Calvert, courtesy of Cetus Corporation

PCR Protocols: A Guide to Methods and Applications

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Michael A. Innis
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A GUIDE TO METHODS AND APPLICATIONS

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PROCEDURES TO MINIMIZE PCR-PRODUCT CARRY-OVER

Shirley Kwok

The ability of PCR to produce large numbers of copies of a sequence from minute quantities of DNA necessitates that extreme care be taken to avoid false positives. Although false positives can result from sample-to-sample contamination, a more serious source of false positives is the carry-over of DNA from a previous amplification of the same target. Because of the large numbers of copies of amplified sequences, carry-over of even minute quantities of a PCR sample can lead to serious contamination problems. The following is a list of procedures that will help to minimize the carry-over of amplified DNA.

Physical Separation of Pre- and Post-PCR Amplifications

To prevent carry-over of amplified DNA sequences, reactions should be set up in a separate room or containment unit such as a biosafety cabinet. A separate set of supplies and pipetting devices should be

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dedicated for the specific use of sett
taken to insure that amplified DNA
Reagents and supplies should be tal
nets and must never be taken from a
being performed. Similarly, devices
be taken into the containment area

Aliquot Reagents

All reagents used in the PCR mus
stored in an area that is free of PC
oligonucleotides used for amplificat
purified in a PCR-product-free envir
quotted to minimize the number of
able to record the lot(s) of reagents u
it can be more easily traced.

Positive Displacement Pip

Contamination of pipetting devices
tion of samples. For example, the
often contaminated with radioisoto
To eliminate cross-contamination c
positive-displacement pipettes are r
those manufactured by Rainin (Micr
and plungers. The units are complet

Meticulous Laboratory Tec

Although carry-over of amplified se
jority of the false positives, cross-c
can also be a factor. Consequently,
only in setting up the amplification

sample handling, from sample collection to sample extraction. The following are additional precautions that should be taken:

1. Change gloves frequently.
2. Quick spin tubes before opening them.
3. Uncap and close tubes carefully to prevent aerosols.
4. Minimize sample handling.
5. Add nonsample components (mineral oil, dNTPs, primers, buffer, and enzyme) to the amplification reactions before the addition of sample DNA. Cap each tube after the addition of DNA before proceeding to the next sample.

Judicious Selection of Controls

First, for use as a positive control, select a sample that amplifies weakly but consistently. The use of strong positives will result in the unnecessary generation of a large amount of amplified sequences. If plasmid DNA containing the target sequence is used as a positive control, it should be substantially diluted. Depending on the detection system used, as few as 100 copies of target will suffice as a positive control. Second, use well-characterized negative controls. The extreme sensitivity of PCR may enable the detection of nucleic acid sequence from a sample that is negative by all other criteria. Third, include multiple reagent controls with each amplification. Because the presence of a small number of molecules of PCR product in the reagents may lead to sporadic positive results, it is important to perform multiple reagent controls. The reagent controls should contain all the necessary components for PCR but without the addition of template DNA. This system has proved to be extremely sensitive in detecting the presence of contaminants, as the absence of exogenous DNA enables the efficient amplification of just a few molecules of contaminating sequence.

Although amplified products are most problematic, other potential sources of contamination/carry-over need to be considered, especially when additional manipulations of the amplified DNA are performed. The cloning of amplified product is a case in point. Often, the amount of target generated from an amplification is insufficient for direct cloning and requires re-amplification of the target. To minimize re-amplification of nonspecific products, the band of interest

is first separated on a gel, excised
seed a subsequent amplification.
potentially result in cross-contam
authenticity of the product. Preca
contamination. For example, gel s
in 1 *N* HCl to depurinate any re
similar device should be used to
cause the surfaces of UV transillu
nated, a sheet of plastic wrap sho
the gel from the surface of the U
trols that have been amplified wi
the sample of interest should no
preparative gel.

The list below highlights oth
nation:

1. Plasmid or phage DNA contair
2. Purified restriction fragment o
3. Dot blot apparatus
4. Microtome blades
5. Centrifuges
6. Speed Vacs/vacuum bottles
7. Dry ice/ethanol baths

Other sources of contaminatio
dures (the preparation of sample
similar care) will most certainly
here will serve as a guide in imple
minimize if not eradicate carry-o